Homeobox Genes and Skin Development: A Review

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Homeobox (HOX) genes are a gene family that encode information critical for the normal embryologic development of many different organisms, including vertebrates. HOX genes encode transcriptional regulatory factors that bind to multiple different genes and thereby determine the developmental fate of a cell. The role of HOX genes in the development of skin is undetermined but, based on information from other organisms and recent experimental data from skin models, it is likely that this class of genes is important for the normal development of skin adnexae, pigmentary system, and stratified epidermis during embryogenesis. The purpose of this review is to briefly summarize what is known about HOX genes and to familiarize the reader with recent insights into how HOX genes may function in skin development.
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The nature of pattern development and transformation of a fertilized egg to an adult organism is one of the central mysteries in embryogenesis. Understanding how information contained in linear strands of DNA orchestrates the three-dimensional structure of the embryo has been a major focus of study for developmental biologists during the last 20 years. Recently, significant progress in elucidating the molecular basis of development has come with the discovery of a class of "master genes" that share a highly conserved segment of DNA, the homeobox genes (HOX genes).

Originally identified at the molecular level in *Caenorhabditis elegans,* but most intensively studied in the fruit fly *Drosophila melanogaster,* HOX genes regulate the action of effector genes, which results in organization and development of limbs and segments in the adult fly. Mutations in HOX genes cause one part of an organism to develop with the characteristics of a different part, such as mutations in the antennapedia (Antp) gene (Fig 1), which cause transformation of antennae into legs, or of second and third legs into first legs. Some of these transformations occur as a result of the loss of function of a homeotic gene, whereas other transformations are due to increased activity of the gene.

Since the description of the *Drosophila antennapedia* complex (ANT-C) and the bithorax complex (BX-C), HOX genes have been described in diverse species, from nematodes to mammals, including humans (reviewed in [1]). Defining the extent of HOX gene family has been accomplished by taking advantage of the highly conserved 183-base pair sequence common to all HOX genes to probe the genome of an organism. Currently, there are 38 human HOX genes organized into four clusters on separate chromosomes, which presumably arose through a process of duplication and subsequent divergence. The recent implication of mutations in HOX genes in several different human diseases *(vide infra)* suggests that this widely conserved gene family plays an important role in morphogenesis in humans. Investigations to date indicate that HOX genes control the developmental fate of a cell by encoding transcription factors (Fig 2) that sequentially turn on or turn off effector genes that determine migration, position, and development of that cell within the organism. Regulation of HOX gene function involves the coordinated action of growth factors and cytokines on HOX gene expression, including auto-regulation and post-translational modification of the protein product itself through phosphorylation and glycosylation.

For normal development of skin, cells from ectodermal, neuroectodermal, and mesodermal lineages must join to form a complex three-dimensional network in a precisely defined spatial and temporal order. Although most of the research of HOX gene function has focused on mutations in limb or central nervous system development, the nature of these genes as transcription regulators and their expression in human skin, as documented by *in situ* hybridization and polymerase chain reaction analysis, suggests that this family of genes may play a role in skin development. This review will familiarize the reader with the structure of HOX genes, their products, and the factors that regulate them and review what is known about HOX gene expression and function in the skin.

HOMEOBOX GENE ORGANIZATION AND EXPRESSION

Prior to the advent of molecular biology and cloning technology, the existence and organization of HOX genes was inferred by observing the effect of homeotic mutations on the development of *D. melanogaster.* Lewis [2] presciently proposed that HOX genes in the BX-C complex are positioned on the chromosome in the same order as their expression at the antero-posterior axial level ("colinearity"). He suggested that the arrangement of the individual HOX genes in the complex on the chromosome resulted in an anterior-posterior gradient in repressor concentration in the segment, which corresponded to the linear arrangement of the genes on the chromosome.

With the ability to clone genes in the absence of biochemical information of their products, several independent groups quickly isolated members of the two HOX gene complexes in *D. melanogaster.* Initially, isolation of individual HOX genes in the complexes was achieved by chromosomal walking and jumping, which resulted in the identification of several genes in the ANT-C and BX-C complexes [3,4]. Present day drosophila have eight homeotic genes located in a linear array in the ANT-C and BX-C gene complexes on the long arm of chromosome 3 (Fig 3). Genes in the ANT-C complex control the development of the head and anterior portion of the fly, whereas genes in the BX-C complex direct the development of the posterior and abdominal segments. Once all the genes in the ANT-C and BX-C complexes had been identified, and their expression boundaries defined, it was shown that the homeotic

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Abbreviations: ANT-C, drosophilia antennapedia complex; BX-C, bithorax complex; HNF, Hepatic nuclear factor; HOX, homeobox.

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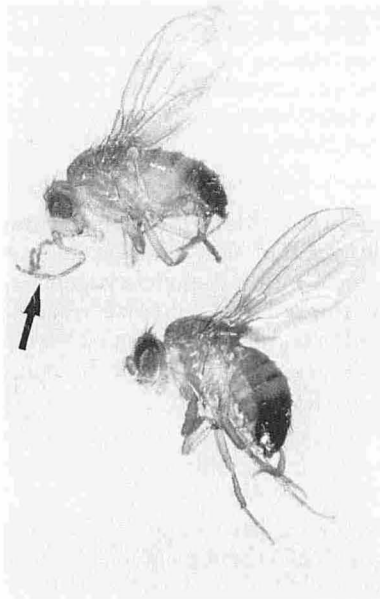

Figure 1. An example of *D. melanogaster* with a mutation in the HOX gene antennapedia (Antp). The normal fly is at the *bottom;* the mutant fly *(top)* has developed legs where antennae used to be (*arrow;* courtesy of Dr. Matthew Scott, Stanford University).

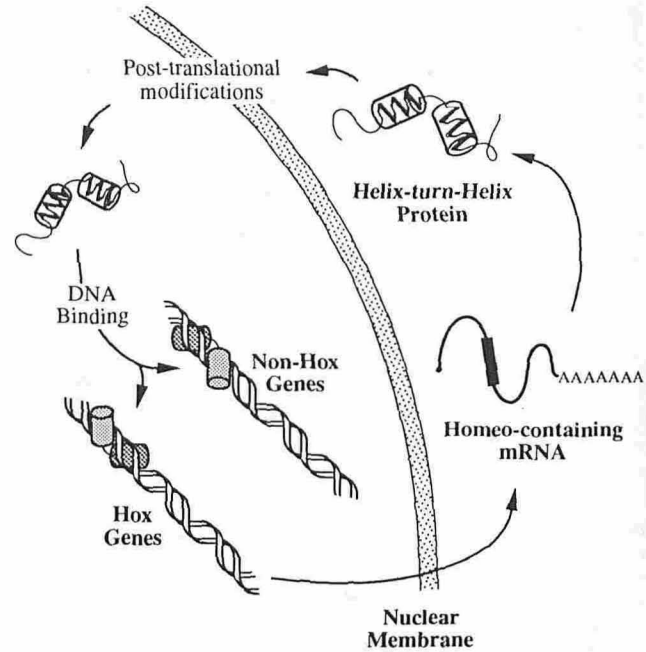

Figure 2. Schematic representation of homeobox gene function. The homeobox gene product is a monomer protein that contains a highly conserved site (the homeodomain), which assumes a helix-turn-helix configuration. After post-translation modification of the protein, it specifically binds to DNA of HOX and non-HOX genes to regulate transcription.

genes were arranged in the same order on the chromosome as their expression domains in the anterior-posterior axis, with the most 3′ gene, *Lab,* expressed most anteriorly. Thus, Lewis's elegant hypothesis of colinearity was proved to be valid.

HOMEOBOX GENE STRUCTURE

Some HOX genes are extremely large, such as *Ubx* and *Antp,* which contain 73 kb and 103 kb, respectively, and encode multiple RNA species, whereas others, such as *Ftz,* are simpler transcription units of about 2 kb. All HOX genes share a highly conserved ("homologous") sequence of about 183 base pairs, which is located in a separate exon near the proximal border of the 3′ exons in transcripts and cDNAs [5]. Homology among HOX genes is striking, as shown with the *Antp* and *Ftz* gene (77% homology), and the *Ftz* and *Ubx* gene (75% homology [5,6]). Even when there are substitutions in the coding sequence between homeoboxes, the changes usually preserve the same amino acid codon.

Using the fly homeobox region as a probe and low-stringency hybridization to vertebrate DNA, investigators quickly identified HOX genes in *Xenopus laevis* [7], mouse [8], and humans [5]. Unlike *D. melanogaster* HOX genes, which are arranged in tandem on a single chromosome, the majority of vertebrate HOX genes are present as four clusters on separate chromosomes. At a recent meeting in Ascona, Switzerland it was determined that all the homeobox gene clusters in humans and mice have been identified, and a new system of nomenclature was adopted (Fig 4 [9]).

HOX Gene Expression in Vertebrates Most of the information concerning HOX gene expression has come from *in situ* hybridization studies on mouse embryos. HOX genes are expressed after primitive streak formation, suggesting that these genes are not involved in the establishment of the main body axis. The general rule that HOX gene expression in the organism corresponds to its location on the chromosome is true for the vertebrate HOX genes, in which the most 3′ genes are expressed anteriorly, and the most 5′ genes are expressed posteriorly (reviewed in [10]). Unlike *D. melanogaster,* however, in which anterior and posterior expression boundaries are clearly delineated, vertebrate HOX genes usually show some overlap in expression, particularly in the posterior expression boundary. *In situ* hybridization studies have shown that the first genes to be expressed are found in the mesoderm and ectoderm of the posterior mouse embryo, with subsequent spreading of expression to more anterior regions. HOX genes are strongly expressed in the CNS and examination of human HOX gene expression by Northern analysis has shown that HOX genes are expressed in an organ-specific and stage-specific pattern in human embryos, indicating that HOX genes may exert a wide spectrum of control in early fetal development [11].

A role for vertebrate HOX genes in limb development has been inferred from the expression pattern of the five genes from the extreme 5′ end of the HOX 4 cluster. These five genes are expressed in a defined temporal order and subdivide the limb bud into five zones. Mapping experiments have shown that regional domains of HOX expression correlate with the anlage of individual digits [12]. Direct proof of the role of HOX genes in limb development was provided by Morgan *et al* [13], who used a viral vector to ectopically express the HOX 4.6 gene in the developing chick hind limb. Ectopic expression of this gene resulted in the transformation of a digit that normally does not express the HOX gene to the morphology of a digit that normally expressed the HOX 4.6 gene. These

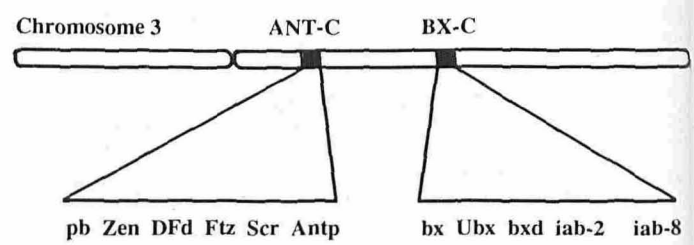

Figure 3. The ANT-C and BX-C homeobox gene cluster of *D. melanogaster* is on the long arm of chromosome 3. There are eight HOX-containing genes arranged in tandem on the chromosome. The location of the gene on the chromosome corresponds to its expression in the anterior-posterior axis (adapted from [5]).

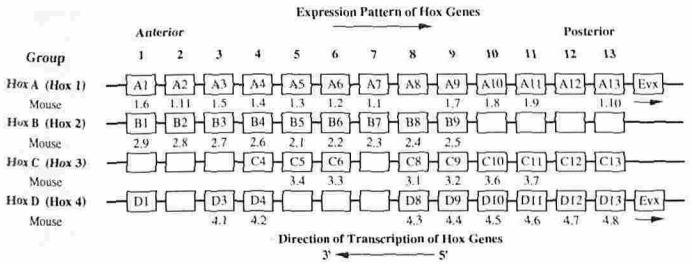

Figure 4. Four mammalian HOX gene complexes and the new and old names of the genes. The new names are single letters (*A*, *B*, *C*, or *D*) followed by a number from 1 to 13. The most anteriorly expressed genes have the lowest numbers, and the names shown are in the order that they are arranged on the chromosome. As in *D. melanogaster,* the location of the gene on the chromosome corresponds to its expression pattern on the anterior-posterior axis ("colinearity"). Each column of genes indicates corresponding genes in the four HOX gene complexes based on sequence identity alone. *Empty squares,* no gene has been detected in mice or humans (adapted from [9]).

results imply that the pattern of HOX gene expression is instrumental in determining the identity of digits.

STRUCTURE AND FUNCTION OF THE HOMEODOMAIN

One portion of the homeobox gene, the homeodomain, is a 60–amino acid subregion of the protein that has been shown to be very highly conserved across different species. The homeodomain is over 10^9 years old, as demonstrated by its presence in proteins of fungi, plants, and animals. The homeodomains of the *Antp, Ftz,* and *Ubx* genes show greater conservation at the amino acid level than at the DNA level, a phenomenon that has been proved to be a general rule for the homeodomain, suggesting that this subregion of the protein has a critical function. Over 87 homeodomain proteins have been identified, and have been placed into sets of 10 classes based on sequence homology [14]. The first class to be identified is referred to as the antennapedia class homeodomains (class I), which are identical to the antennapedia homeodomain at more than 50 of 61 amino acids. The fact that most classes contain homeodomains from several different species suggests that common features of the group have been evolutionarily conserved.

The predicted amino acid sequence of the homeodomain shows that about 30% of the amino acids are basic, suggesting a DNA-binding function for the protein. Another clue to the function of the homeodomain came when Shepherd *et al* [15] showed that the homeodomain shares significant homology with the amino acids coded from parts of the α1 and α2 yeast mating type proteins. The mating type α1 and α2 genes code for an α-helix-turn-α-helix sequence that act as transcription factors to regulate the expression of many unlinked genes that control cell differentiation. The homology between the homeodomain and the mating type proteins suggested a similar role for the homeodomain as a transcriptional factor. The localization of homeobox proteins to the nucleus adds further support for a function as a transcriptional regulator.

Direct evidence for the helix-turn-helix structure of the homeodomain was provided by nuclear magnetic resonance studies of the purified protein, which showed that the homeodomain folds into three α helices (I, II, and III), with the second two adopting a helix-turn-helix motif [16]. The DNA binding function is contained in the third (recognition) helix, which forms hydrogen bonds and van der Waals contacts with the DNA backbone of the target sequence. In fact, the most variable positions in the recognition helix of the homeodomain, the first, second, fifth, sixth, and ninth positions, are the same amino acids predicted from bacterial DNA binding proteins to contact DNA. DNA binding studies of purified homeodomains to various DNA sequences revealed that homeodomains recognized specific sequences within the DNA *(vide infra)*. Desplan *et al* [17] showed that the drosophila engrailed (En) homeodomain, with only a small amount of surrounding En protein, is capable of DNA binding; studies of mammalian homeodomain have also shown sequence specific binding. DNA footprinting and immunoprecipitation studies have better defined the DNA consensus sequence and targets of homeodomain proteins, revealing that homeodomains bind to other HOX genes, such as the drosophila evenskipped (Eve) homeodomain, which binds specifically to sequences near the 5′ end of the engrailed gene, and to themselves.

Because all homeodomain proteins share the same basic secondary structure, specificity of DNA binding may reside in individual amino acids at specific critical sites in the recognition helix. This was demonstrated to be the case by Treisman *et al* [18], who showed that a single amino acid confers binding specificity to the Paired (Prd) class of homeodomain proteins. They accomplished this by altering specific amino acids in the *Prd* homeodomain, and then analyzed the DNA binding specificities of the wild-type and mutant protein. Substitution of serine for glutamine at position 9 of the recognition helix, which is contained in the *Ftz* homeodomain, resulted in a change in DNA binding specificity from the *Prd* wild-type target to the *Ftz* target. Similarly, when a lysine occupied this position, as in the bicoid (Bcd) protein, the Prd mutant recognized the Bcd recognition sites. Although the sequence of the recognition helix is of importance in determining the regulatory specificity of the protein, it cannot be the only important region, because homeodomains with identical amino acid sequences in the regulatory helix are known to recognize different targets. This was demonstrated by Kuziora and McGinnis [19], who replaced the homeobox of Deformed *(Dfd)* with the homeobox of *Ubx*. The resulting chimeric protein failed to recognize the normal target of the *Dfd* homeobox, but instead activated ectopic transcription of *Antp,* a gene normally regulated by *Ubx*. Because the *Dfd* and *Ubx* homeoboxes are identical, this implies that target specificity may be determined by factors other than the nature of the recognition sequence.

FUNCTION OF HOMEOBOX GENES

Genetically altered HOX genes and their target genes may be used as precise probes for determining HOX gene function. Until recently, spontaneous mutations and chemical and irradiation-induced mutations were the major sources of such genetic models; recently, recombinant technology has allowed scientists to alter portions of HOX genes, or the entire gene, enhancing the power of such genetic analysis.

Spontaneous Mutations Much of the work on the effect of HOX gene mutation on structure and function of the developing organism has been performed on the POU (pronounced "pow") class of HOX genes. The POU genes are a family of homeodomain genes with a second conserved DNA-binding motif, in addition to a homeobox (termed a "paired box"). Because many POU target genes are well characterized and encode known proteins with well-described functions, analysis of these genes has proved very useful for understanding HOX gene function. In humans, an autosomal dominant point mutation in Pit-1, a transcription factor within the POU family, is associated with profound growth retardation, severe mental retardation, and deficiency of growth hormone, prolactin, and thyroid-stimulating hormone [20]. The point mutation has been localized to the POU homeodomain, which results in a substitution of Arg with Trp at a site implicated in DNA binding. Mutations have been found in both sporadic and familial cases of the disorder. The mutation is classified as dominant negative because it interferes with the activity of the wild type allele, i.e., the affected individual has less than 50% of the normal gene product. A similar mutation of Pit-1 in dwarf mice results in the absence of lactotroph, somatotroph, and thyrotroph cells in the pituitary and leads to the absence of expression of a G-protein–coupled receptor in pituitary cells [21] and illustrates the cascade of effects resulting from a single point mutation in a HOX gene.

Mutations in HOX containing genes have recently been implicated in the diseases Waardenburg syndrome, aniridia and the Wolf-Hirschhorn syndrome. Waardenburg syndrome accounts for

about 2% of cases of adult deafness and has associated facial irregularities and pigmentary abnormalities such as a white forelock and heterochromia iridis. The Splotch (Sp) defect in mice is the murine homologue of Waardenburg syndrome, and is due to a dominant mutation in the Pax-3 gene, a member of the paired-box family (class) of HOX genes. Recently, mutations in the PAX 3 gene have been implicated in the expression of the WS phenotype in humans [22,23]. In aniridia in humans, a candidate gene for the disease has been identified that contains a paired domain and a homeodomain, and in two patients deletions of this gene have been identified [24]. Mice with the autosomal dominant mutation small eye (Sey), a homologue of the human disease aniridia, have a point mutation that results in the insertion of a stop codon upstream of the wild-type stop codon. The mutant protein lacks the homeobox region of this paired-like homeobox gene [25]. Finally, Wolf-Hirschhorn syndrome (a midline fusion syndrome with delayed growth and development and congenital abnormalities), which maps at chromosome 4p16.1, has been associated with the HOX 7 locus, which maps to the same location [26]. The potential role of the HOX genes and congenital malformations has been recently reviewed [27].

Knock-Out Experiments In addition to these spontaneous mutations, knock-out experiments have been used to establish the functional role of specific homeobox genes in transgenic mice. In these experiments, genes or portions of genes are removed from embryonic stem cells, which are then introduced into fertilized mouse ova forming transgenic mice. Using this approach, expression of an altered mouse HOX 1.5 gene resulted in mice with a syndrome resembling human Di George's syndrome (absent thymus, parathyroid glands and hyoid bone, reduced thyroid and submaxillary tissue, craniofacial abnormalities, and defects in the heart including aortic stenosis [28]). Heterozygotes were normal, but the trait was lethal in homozygotes, suggesting that a single dose of the normal gene is able to sustain a normal program of differentiation and that the abnormal gene did not function as a dominant negative mutation.

In addition to analysis of HOX gene deletions, transgenic mice have been constructed that ectopically express the product of the HOX 4.2 gene, a gene normally expressed in the cervical vertebrae. Ectopic expression of this gene in the occipital bone results in development of the occipital bone with characteristics of cervical vertebrae. These results suggest that the HOX 4.2 gene is able to alter the program of differentiation when expressed in an alternative site [29].

REGULATION OF HOMEOBOX GENE FUNCTION

Homeobox proteins primarily function as transcriptional regulatory factors [30]. Elucidating the exact nature of the interactions between HOX proteins and target DNA, other HOX proteins, and the molecules that regulate transcription allows determination of the detailed molecular functioning and control of the HOX network. Characterizing the effects of hormones, vitamins, growth factors, and cytokines on the HOX proteins places them in a more physiologic context. The relationships of HOX proteins structure and function across species adds the dimension of evolutionary time into a model for HOX function. In this section, data from humans, when available, will be emphasized.

HOX genes function primarily through regulating the transcription of other genes, including gene cascades. In drosphilia many of the target genes are other HOX genes that are expressed in the imaginal discs, spinal cord, or visceral mesoderm. Some of the genes directly regulated by HOX include auto and alloregulation of the HOX themselves [31,32], extracellular signaling molecules such as cytotactins [33], cell surface molecules such as connectins, and the serum response family of proteins. HOX genes have also been shown to regulate genes that have profound effects on the growth and differentiation of multiple cell lines, such as the decapentaplegic gene, a member of the transforming growth factor-beta gene family. The POU family of HOX proteins regulate the pituitary releasing hormones as discussed above.

The target sequences in DNA recognized by the HOX proteins have been identified and are usually a group of 10–12 base pairs around a central TAAT motif. HOX proteins regulate their own transcription and the transcription of other HOX genes by binding to one or several different consensus sequences on the gene. Other sequences including a consensus sequence TCAATTAAAAT [18] and a 217-bp fragment containing two TAAT motifs are recognition sites for HOX-containing proteins [34].

Regulatory Interactions HOX genes can be either inducers or repressors of target gene function. This bi-directional effect is not related to the nature of the target sequences but to differences in interactions with other transcription factors. HOX genes are regulated through several mechanisms, including post-translational modifications such as phosphorylation or glycosylation, which may in turn be functioning by regulating the dimerization of HOX proteins. The monomers and dimers may have different affinities for other transcription factors, or DNA itself.

Interactions of HOX proteins with other transcriptional regulators is a general mechanism for the regulation of homeoprotein function. Heterodimerization and homodimerization are mechanisms of regulation for hepatic nuclear factor (HNF1 [synonymous with HNF1-alpha]), which is a homeoprotein that regulates transcription of a number of hepatic proteins [35]. HNF1 binds to the promoter sequences of over 20 proteins including albumin, alpha-fetoprotein, and alpha and beta-fibrinogen. HNF1 binds efficiently to DNA only as a dimer, which differs from other vertebrate homeoproteins, which bind to DNA as monomers. The affinity of binding of HNG-1 to its target DNA is regulated, in part, by the co-factor DCoH, which facilitates dimerization of HNF-1 and therefore increases binding to its recognition sequences [35,36]. Other regulatory factors have been reported: phosphorylation and glycosylation [37]; topoisomerase II, which has been shown to regulate HOX 2.1 transcription [38]; and Krpx-20, a zinc-finger gene product, which binds to DNA sites upstream of HOX-1.4 [39].

RETINOIDS AND HOMEOBOX FUNCTION

Retinoic acid (RA) is an important morphogen during several stages of development and has been implicated in the control of several HOX genes; further, the teratogenic effect of vitamin A has been directly linked to the activation of some HOX genes *(vide infra)*. In the F9 mouse teratocarcinoma cell line, RA induces an early response protein, Era-1, which contains the HOX 1.6 domain [40]. Era-1 then initiates a process that leads to the production of a large number of matrix proteins and other molecules. In a human embryonal cell line, RA causes sequential activation of the HOX-2 gene cluster with the 3′ genes in the complex being activated before the 5′ genes [41]. Genes at the 3′ end are activated at lower concentrations of RA than genes at the 5′ end of the cluster. Retinoic acid has been shown to induce HOX 1.6 transcription via a response element for the RAR-β receptor, which is present in the 3′ region of the HOX 1.6 gene. The HOX 1.6 gene in turn regulates HOX 2.9 gene expression, indicating that RA may induce a gene cascade. In a recent report, Cheng-Ming *et al* showed that RA changes the gradient of the XIHOX 1 homeoprotein in developing chick feather buds, resulting in a more diffuse pattern of expression, rather than an anterior-posterior gradient [42]. They suggest that modulation of HOX gene expression by RA may be a mechanism for the alteration in orientation or phenotype seen in skin appendages treated with RA. The action of RA on HOX genes is specific for certain HOX genes that are expressed at defined stages during development, because some HOX genes are neither up- nor downregulated by retinoids [43]. In addition to direct transcriptional control, RA can regulate HOX gene function through post-transcriptional activation of HOX proteins [44]. The complexities of interactions that are possible is illustrated by HOX 3D, which has upstream binding sites for RA-induced nuclear factors, its own product (HOX 3D),

and another HOX protein HOX 3B [45]. In the mouse, the type I and type II keratin gene clusters, are each related with a HOX cluster and a RA response gene. The functional significance of this association, albeit intriguing, is unknown [46].

Retinoic acid and HOX genes are both involved in the normal development of craniofacial structures, suggesting that RA may alter the expression or function of the HOX genes expressed in this site. The application of RA to mouse embryos at specific embryonic stages results in the ectopic expression of genes located at the 3′ end of the HOX 2 gene cluster (HOX 2.9, HOX 2.8, HOX 2.6, and HOX 2.1 [47]). When transgenic mouse with lacZ reporters for following endogenous HOX B1 (HOX 2.9) and HOX B2 (HOX 2.8) and Krox-20 gastrulas are treated at the pre-headfold stages with retinoic acid, rhombomeres of the hind brain can be altered and multiple components of the first branchial arch are transformed, indicating that normal migration of neural crest elements has been affected [48].

HOMEOBOX EXPRESSION IN THE SKIN

Skin is a complex multicellular organ in which the endoderm, neural crest, and ectoderm contribute to form a three-dimensional unit in a spatially and temporally defined manner. Because skin morphogenesis involves the action of multiple genes in a coordinated fashion, it is likely that HOX genes play an important role in skin development. If HOX genes do act as transcriptional regulators in the skin, an important property would be that they are expressed in a spatially and temporally restricted manner, such that a "field" or "gradient" is established. The chick feather bud is an analog of the developing hair follicle, and recent studies in the feather bud using an antibody raised against a HOX gene homologous with the HOX C6 in humans, revealed that a HOX gene and its protein are distributed in a gradient in the mesoderm, with the strongest expression in the anterior-proximal region. The epithelial layer over the feather bud was also positive for HOX protein expression, but no obvious gradient was observed [49].

The expression of HOX genes in murine hair development has been studied by Bierberich *et al* [50], who used reverse polymerase chain reaction analysis on skin samples to show that the HOX 3.1 gene is expressed by murine skin and that the level of expression was greater in skin from the posterior sample than from the anterior sample. To address the question of the cellular localization of the HOX 3.1 gene, Bierberich *et al* used transgenic mice in which the transgene consisted of the cis-regulatory region of the HOX 3.1 gene cloned into the *Escherichia coli* β-galactosidase gene as a reporter. Full-thickness biopsies from the adult animals incubated with the chromogenic substrate for β-galactosidase revealed that the β-galactosidase product is localized to the dermal papillae of the anagen hair follicle, suggesting that the native HOX 3.1 gene may be expressed in this population of cells. Examination of skin sections from the dorsal and ventral trunk showed that β-galactosidase product has a graded distribution, with the strongest activity observed posteriorly. These results suggest, but do not prove, that the HOX 3.1 gene is expressed in a graded pattern in the skin, and may be involved in epithelial-mesenchymal interactions of the hair. Further analysis of the role of HOX 3.1 in hair and skin development must await *in situ* localization of the HOX 3.1 gene product.

A very limited amount of work has been performed on HOX gene expression in human skin. Thomas *et al** isolated a HOX gene from a cultured human keratinocyte cDNA library and showed that it contained a HOX gene homologous to the mouse HOX 1.1 gene. The gene mapped to human chromosome 7, which is the site of the human HOX 1.1 cluster, and showed 100% amino acid homology within the HOX region with the murine HOX 1.1 and 94% nucleotide homology. *In situ* hybridization of 15-week gestational age fetal skin showed that the gene was expressed predominantly within the epidermis, but not in the hair follicle epithelium. In conjunction with Dr. Kurt Stenn at the Skin Biology Research Center of Johnson and Johnson Pharmaceutical Research Institute, we have preliminary data to show that cultured human fetal melanocytes express several members of the HOX A and C families, including A.1, A.4, A.6, C.2, and C.3. Interestingly, overexpression of HOX A.1 (murine HOX 1.1) in transgenic mice results in abnormalities in neural crest–derived cranio-facial structures, such as cleft palate, open eyes at birth, and non-fused pinnae [51], and overexpression of HOX A.4 results in megacolon, presumably from a failure of migration of enteric ganglion cells, which are also neural crest derivatives [52].

These early studies show that HOX genes exhibit spatial and temporal expression in the skin, suggesting a role for these molecules in skin morphogenesis. The exact role of HOX genes in the development of skin must await further studies on the expression, target sequences, and regulatory interactions of HOX genes with growth factors and other molecules important for skin morphogenesis. Based on information about the function of these genes in the development of *D. melanogaster* and vertebrates, however, it appears that these master genes coordinate the three-dimensional organization of many different tissues, including the skin. Processes such as wound healing, hair follicle transition from a telogen to anagen, and diseases with markedly altered differentiation such as psoriasis and pityriasis rubra pilaris are physiologic states in which HOX gene function may be important in adult life, and therefore this field should provide new insights into skin development in the future.

Note Added in Proof: Since submission of this manuscript, several HOX genes have been shown to be expressed in developing mouse fetal epidermis and human squamous carcinoma cell lines, but not in normal cultured human keratinocytes (Detmer K, Crumrine D, Largman C. J Invest Dermatol 100:525A, 1993). In addition, further studies have questioned the nature of the relationship between the HOX gene H6 at 4p16.1 and the Wolf-Hirschorn syndrome (Standler HS, Padanailam BJ, Buetow K, Murray JC, and Solursch M. Proc Natl Acad Sci USA 89:11579–11583, 1992).

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